Journal of Experimental Microbiology and Immunology (JEMI) Vol 14: 85-91
Copyright © April 2010, M&I UBC

The Effect of Cell Size on the Burst Size of T4 Bacteriophage Infections of *Escherichia coli* B23

Charles Choi, Eugene Kuatsjah, Elizabeth Wu, and Serena Yuan
Department of Microbiology and Immunology, UBC

Previous experiments have shown that individual bacteriophage infections of *Escherichia coli* cells have burst sizes ranging from a few phage to hundreds of phage. It was proposed that larger cells have more cellular machinery and can therefore support higher rates of phage production resulting in larger burst sizes. The effect of cell size on the ability of T4 bacteriophage to replicate and lyse from *E. coli* cells was investigated. *E. coli* B23 cells were separated to size by centrifugation using 30% (w/v) sucrose and the single burst experiment was performed on the bacteria extracted from the top and bottom of the bands after centrifugation and on a mixed sample of mid-log phase *E. coli* B23 cells. *E. coli* B23 cells were successfully separated by size and results of the single burst experiment indicate that cell size has an effect on the burst size resulting from T4 bacteriophage infection of *E. coli* B23 cells.

Ever since the discovery of bacterial viruses by Twort and d`Herelle, bacteriophages have been of great interest in bacteriology (9). Bacteriophages have served as a tool for understanding molecular biology and as a potential treatment agent to combat bacterial infection. Great insight into bacteriophages came from the results of a series of experiments by Ellis and Delbrück. One of their most noted experiments on bacteriophages is the one step growth curve. This experiment is a clear demonstration of how the production and release of phage progeny occurs after a subset of time post infection. This experiment showed the average number of phage progeny released from infected cells after a single round of infection. This number is also known as the average burst size. In order to examine this differential burst size phenomenon, Ellis and Delbrück devised a single burst experiment to observe the actual number of phage progeny released by an infected cell. Delbrück found that infection of a single strain of *E. coli* with a single type of bacteriophage could lead to burst sizes ranging from 20 to over 1000 pfu per cell (2).

There are many possible explanations for this vast range in burst size variation for a single infection. It was originally proposed that since a single bacterium can adsorb many phage particles; bacteria that were infected with multiple phages would have an increased burst size because more phage would be present at the start of infection (2). However, Ellis and Delbrück found that bacteria that were infected by multiple phages had similar burst sizes as the bacteria that were infected with only one particle (3). Ellis and Delbrück also found that the time of the bursts had no effect on burst size (3). Faster growing *E. coli* cells are larger and have proportionally more surface area to which phage can attach and be adsorbed (6). Density of bacteriophage receptors does not change with cell dimensions (6). However, in larger cells with more surface area, there will be more receptors available to phage compared to a smaller cell with less surface area. In addition, larger cells have a higher proportion of protein synthesis machinery than smaller cells due to the difference in cell volume (6). This would potentially enable a greater number of virus particles to be replicated leading to a larger burst size (6). Most of the above factors can be attributed to the size of the cells. Thus, the purpose of this experiment was to investigate the effects of cell size on the burst size of bacteriophage infections of *E. coli*.

Density gradient centrifugation is a technique that separates particles based on density and size. It is a direct application of Stoke's equation (8), which states that the sedimentation velocity of a particle is directly proportional to the volume of the particle and the difference between particle density and gradient density. In addition, it is inversely proportional to the radius of the particle. This technique is typically used for separation by density, but separation by size can be achieved if the particles in the sample are of equal density. Since velocity is directly proportional to cell volume, the smaller cells will travel slower through the gradient and reside in the upper region of the broad band obtained (12).

Copyright © April 2010, M&I UBC

MATERIALS AND METHODS

Bacterial and Phage Strains. T4 phage was chosen for this experiment because the development of T4 phage in *E. coli* is well documented and T4 phage is commonly used in the study of bacteriophage infections of *E. coli*. *E. coli* B23, T4 bacteriophage, and wild-type *Bacillus subtilis* WB476 were provided from the MICB 421 culture collection of the Department of Microbiology and Immunology of the University of British Columbia. *E. coli* B23 and *B. subtilis* WB476 were maintained on H agar (tryptone (10.0 g), sodium chloride (8.0 g), agar (15.0 g), distilled water (to 1 L), pH > 6) and Luria-Bertani (LB) agar (tryptone (10.0 g), sodium chloride (5.0 g), yeast extract (5.0 g), agar (15.0 g), distilled water (to 1 L), pH > 6) plates respectively. T4 bacteriophage was grown from the original laboratory stock and maintained in H broth (tryptone (10.0 g), sodium chloride (8.0 g), distilled water (to 1 L), pH > 6).

Cell culture. An overnight culture of *E. coli* B23 was prepared by inoculating H broth with a single isolated *E. coli* B23 colony from an H plate and incubating it in a 37°C shaking water bath. For cultures grown to mid-log phase, 50 ml of fresh H broth was inoculated with the appropriate amount of an overnight *E. coli* B23 sample to achieve an OD_{660} between 0.10 and 0.15, and incubated at 37°C until the OD_{660} reached 0.6.

***E. coli* B23 growth curve.** Turbidity readings of an overnight *E. coli* B23 culture were taken using a spectrophotometer set to 660 nm. The turbidity of the culture was adjusted to a final OD_{660} of 0.10 to 0.15 using H broth and incubated at 37°C with constant aeration. At half hour increments up to 2 hours, a turbidity reading was taken and the samples were diluted in sterile saline (sodium chloride (9.0 g), distilled water (up to 1 L)) to the appropriate concentration for spread plating on H agar plates in duplicates. Plates were incubated overnight at 37°C. A growth curve was made using the results of the plate counts to determine the incubation time required and approximate OD_{660} for the *E. coli* B23 culture to achieve mid-log phase.

***E. coli* B23 OD_{660} to cell concentration conversion factor.** The turbidity of an overnight *E. coli* B23 culture was adjusted to a final OD_{660} of 0.3, 0.4, and 0.5 using H broth. Samples were diluted in sterile saline to the appropriate concentration for spread plating on H agar in duplicates. Plates were incubated overnight at 37°C.

Concentration determination and preparation of killed *B. subtilis* WB476. Turbidity readings at 660 nm of an overnight *B. subtilis* WB476 culture were taken and six series of 10 fold dilutions up to 10^{-7} were prepared using sterile saline. Then, 0.1 ml of each dilution was plated in duplicates on LB plates, incubated at 37°C overnight and then counted. The overnight *B. subtilis* culture was then killed by heating for 15 minute at 60°C and stored at 4°C.

Phage stock preparation. This method was adapted from the textbook, Molecular Biology of Bacteriophage T4 (7). H top agar (tryptone (10.0 g), sodium chloride (8.0 g), agar (480 g), distilled water (up to 1 L), pH > 6) was melted in a microwave and maintained in a liquefied state in a 50°C water bath. The supplied T4 bacteriophage stock was diluted using 10 fold serial dilutions in H broth up to 10^{-10}. Then 0.1 ml of each serial dilution and 0.5 ml of overnight *E. coli* B23 indicator culture was added to sterile glass test tubes. The tubes were left to stand 5 minutes at room temperature to allow for adsorption. Subsequently, 3.0 ml of H top agar was added to each tube and the contents of the tube were mixed by phage style mixing. The contents of the tube were poured onto H plates and left to solidify for 5 minutes. Plates were incubated at 37°C overnight. The next day the plates were observed and the plate with one 10 fold dilution above the plate with complete lawn clearance was chosen. This plate was flooded with 3 ml of H broth and the top agar was scraped with a sterile glass spreader and spatula. The top agar along with the broth was transferred into a Teflon FEP Oak Ridge tube and 0.1 ml of chloroform was added. The tube was left at 4°C overnight. The tube was centrifuged at 5,000 x g for 10 minutes in a JA-20 fixed-angle rotor in the J2-21 centrifuge (Beckman Instruments Inc.), and the aqueous phase (phage stock solution) was carefully pipetted into a sterile glass test tube and stored at 4°C.

Phage titer determination. This method was adapted from the textbook, Molecular Biology of Bacteriophage T4 (7). Ten fold serial dilutions up to10^{-9} of the prepared T4 bacteriophage stock solution were prepared using H broth. Phage style mixing was used to mix each of the serial dilutions. Duplicates of 0.25 ml, 0.05 ml, and 0.10 ml of the 10^{-9} dilution were each mixed with 0.25 ml of *E. coli* B23 overnight culture and 3 ml of H top agar by phage style mixing. Each mixture was poured onto H plates and incubated at 37°C overnight and counted the next day.

Cell separation by size. Five millilitres of the mid-log phase culture was centrifuged at 10,000 x g for 2 minutes, decanted, and resuspended in any residual supernatant. These cells were layered onto a 30% (w/v) sucrose solution in a 13 X 51mm Quick-Seal® polyallomer centrifuge tube (Beckman Instruments Inc., Part No. 342412), placed into the JA-20 fixed-angle rotor (Beckman Instruments Inc.) with custom-made tube adapters, and centrifuged for 5 minutes at 10°C and 2000 rpm in the J2-21 centrifuge (Beckman Instruments Inc.). Using a sterile syringe, 0.3 ml of cells from the top and the bottom of the resulting band were removed, placed into Eppendorf tubes, and put on ice. Sucrose was removed by resuspending the cells with 1 ml of sterile saline and centrifuging at 10,000 x g for 2 minutes. The sample was decanted and the pellet was resuspended with 1 ml of sterile saline.

Sample characterization of size separated samples. The cell concentration of the smaller cell fraction (from top band), larger cell fraction (from bottom band), and mixed sizes (mid-log phase culture) was determined by direct counting using Petroff-Hausser counting chambers and a phase contrast microscope. The counts from 16 squares were averaged to determine the sample concentrations. All samples were adjusted to the same concentration. To confirm the separation of *E. coli* B23 cells based on size, digital photographs of Gram stains of mixtures of the smaller cell fraction and larger cell fractions with the killed *B. subtilis* WB476 were taken. These images were blown up using a computer and the *E. coli* B23 to *B.subtilis* WB476 length ratios were determined for the smaller and larger cell fractions. To further confirm that cells were separated based on size 0.1 ml of each sample were serially diluted using H broth. Appropriate dilutions were plated in duplicate on H agar plates. Then, another 0.1 ml of each sample were mixed with 9.9 ml of H medium and incubated at 37°C for 30 min. in a shaking water bath. The density of the mid-log phase culture was characterized via centrifugation using Percoll. Five millilitres of the mid-log phase culture was centrifuged at 10,000 x g for 2 minutes, decanted, and re-suspended in any residual supernatant. These cells were layered onto a Percoll suspension (7.5 part v/v Percoll® (Sigma-Aldrich, Inc.), 1 part v/v 1.5 M sterile NaCl, and 1.5 part v/v distilled water) in a 13 X 51mm Quick-Seal® polyallomer centrifuge tube, placed into the JA-20 fixed-angle rotor with custom-made tube adapters, and centrifuged for 5 minutes at 10°C and 2000 rpm in the J2-21 centrifuge.

Effect of sucrose on *E. coli*. Five millilitres of the mid-log phase culture was concentrated by a factor of 10 by high speed centrifugation and mixed with 4.5 ml 30% (w/v) sucrose solution for 10 minutes. A separate 5 ml was also concentrated and mixed in sterile saline. Then, 0.3 ml of the suspensions were extracted, washed by 1 ml of sterile saline (centrifuged at 10,000 x g for 2 minutes), and the pellet re-suspended with 1 ml sterile saline, separately. Microscopic counts were used to determine the cell concentration by using the *B. subtilis* WB476 stock with known concentration. Both samples were then normalized to the same concentration by diluting in sterile saline and plated on H agar and incubated at 37°C overnight to determine the cell concentration by plate count.

Single cell burst experiment. T4 bacteriophage from the prepared phage stock were added to 0.4 ml of the normalized Smaller cell fraction, Larger cell fraction, and Mixed cells to achieve an MOI

Copyright © April 2010, M&I UBC

of 0.7. Samples were mixed by phage style mixing and incubated for 10 minutes at room temperature to allow for phage adsorption. The infected culture was diluted with H broth such that there was 0.4 infected cell per 0.5 ml. Forty aliquots of 0.5 ml of each diluted sample were placed in separate test tubes. One drop of chloroform was added to each of the 40 aliquots of an additional mixed sample to check the number of unattached phage. The unattached phages are resistant to chloroform so they will form plaques. The initial phage infected cells will die so they cannot form plaques. The test tubes were incubated for 60 minutes in a shaking water bath at 37°C. Next, 0.20 ml of overnight *E. coli* B23 culture was mixed in each test tube by phage style mixing and let stand for 10 minutes at room temperature. Three millilitres of H-top agar was then added, mixed by phage style mixing, and poured onto an H plate. Once the H-top agar was hardened, plates were placed in a 37°C incubator overnight.

Poisson distribution. The Poisson distribution is a random distribution of independent event occurrences over a specific interval. The Poisson probability function is given by:

$$p_x = P(X = x) = \frac{e^{-\lambda}\lambda^x}{x!}$$

,with the expected number of occurrences λ as the sole parameter. By setting $\lambda = -\ln(p_0)$, where p_0 is the percentage of plates with zero plaques, Stent used the Poisson distribution for determining the number of plates that represent a single burst (9). In this experiment, the expected concentration was 0.4 infected cell per aliquot for a total of 40 aliquots. Then $\lambda_{expected} = -\ln(0.6)$ and the expected number of plates containing a single burst and multiple bursts were 12 and 4, respectively.

Mann-Whitney U test. The Mann-Whitney U test is a statistical test for comparing distributions of groups within a set of data. An advantage of the Mann-Whitney U test is that it is a non-parametric test; thus, it only requires random distribution as an assumption. In this experiment, the burst sizes of the cell size groups are ranked from largest to smallest for calculation of a U test statistic (11).

RESULTS

Effect of sucrose on *E. coli*. The microscopic cell count for the sample of non-size separated cells from the *E. coli* mid-log phase culture (Mixed cell sample) was $6.57x10^8$ cells/ml, and the Mixed cell sample with 10 minute sucrose co-incubation had a concentration of $6.08x10^8$ cells/ml. Also, from plate counts, the Mixed cell sample and Mixed cell sample with sucrose co-incubation had cell concentrations of $1.12x10^8$ cfu/ml and $1.20x10^8$ cfu/ml, respectively. Therefore, we concluded that the 5 minutes of centrifugation with sucrose had no effect on the viability of *E. coli*.

***E. coli* cell separation by size.** A key component of density gradient centrifugation is the gradient solution. Percoll is a high molecular weight, non-toxic colloidal suspension of polyvinylpyrrolidone-coated silica particles that is regularly used for separation of mammalian and bacterial cells (4). Small molecular weight molecules, such as sucrose and salts, are typically used for separation of molecules instead of intact cells due to their high osmotic potentials. However, centrifugation, the Percoll suspension yielded a thin band of *E. coli* cells. Therefore, the cells from the mid-log phase culture had uniform density. On the other hand, the 30% (w/v) sucrose solution yielded a band with significant smearing (data not shown). Thus, sucrose gave a better separation and samples were extracted from that solution. The *E. coli* cells recovered from the bottom of the band (Large cell fraction) had a mean length ratio to *B. subtilis* of 1.30, and the *E. coli* cells recovered from the top of the band (Smaller cell fraction) had a mean length ratio of 0.48. The width ratios of the *E. coli* cells remained constant throughout both samples (Fig. 1). The two mean length ratios were statistically significant at $P < 0.05$ by the two-sample T test (Fig. 2); therefore, the mid-log phase *E. coli* B23 cells were successfully separated by size. In addition, after incubation at 37°C with aeration for 30mins, the Larger cell fraction had a 2.87-fold increase and the Smaller cell fraction had a 1.13-fold increase in *E. coli* cell concentration (Table 1). The greater increase in the

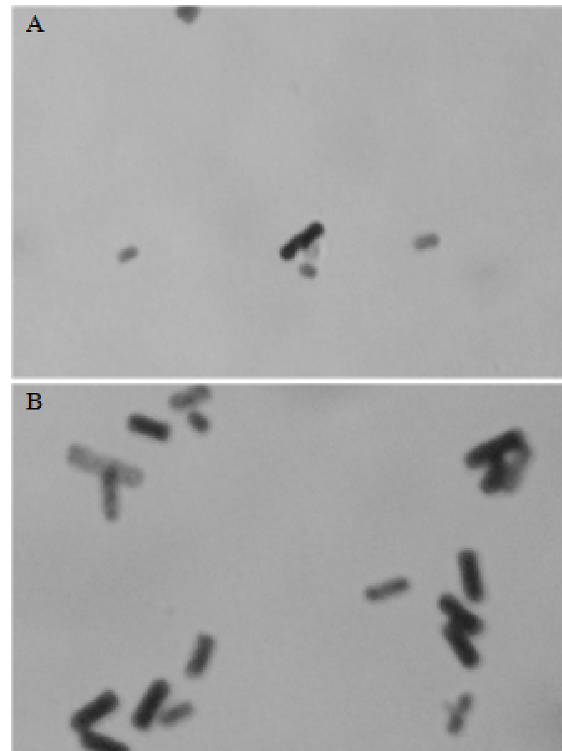

FIG. 1. Gram stain microscopy of *E. coli* and *B. subtilis* cells. Solid black rods are the Gram(+) *B. subtilis*; gray rods are the Gram(–) *E. coli*. (A) contains *E. coli* cells from the Top sample; (B) contains *E. coli* cells from the Bottom sample. Slides were viewed by light microscopy at 1000X magnification; (A) and (B) are not to scale.

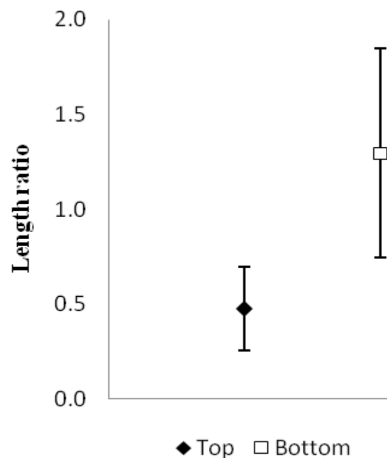

FIG. 2. Comparison of *E. coli* cell sizes in samples recovered from different positions relative to the band after density gradient centrifugation. Length ratio determined by dividing the measured *E. coli* length by the average measured *B. subtilis* length from the same photo (see Fig. 1). The *E. coli* cells from the Top sample had a 95% confidence interval of (0.25, 0.70) length ratio; the Bottom sample (0.75, 1.85) length ratio.

extracted bottom sample was consistent with having larger cells that were closer to division at the time of incubation.

Single burst experiment. The purpose of the chloroform-treated sample was to demonstrate the outcome of having zero infected cells per aliquot. The Larger cell fraction had results identical to those of the chloroform control sample (Table 2); therefore, none of the aliquots from the Bottom sample showed a single T4 bacteriophage burst. Using the Poisson distribution with corresponding p_0 values, the expected number of plates containing a single burst were 7 and 9 for the Smaller cell fraction and the Mixed cell sample, respectively. By comparing the observed number of plates in Table 2, it was assumed that each plaque-containing plate represents a single burst and that the number of plaques corresponds to the burst size. The Smaller cell fraction had a mean burst size of 12 pfu, and the Mixed cell sample had a mean burst size of 15 pfu (Fig. 3). The burst size distributions in both samples were not normal, and the standard deviations were unequal. Thus, parametric tests could not be used to determine the statistical significance of the numerical values. However, the difference was statistically significant at $P < 0.05$ by the Mann-Whitney U test; therefore, the two samples had different distributions. While the Larger cell fraction yielded no significant results, the Mixed cell sample had a larger average burst size than the Smaller cell fraction. Thus, the

TABLE 1. Relative increase in colony forming units when *E. coli* samples recovered from different positions within the band after density gradient centrifugation were incubated for 30 minutes at 37°C. The Mix sample contains non-size separated cells from the *E. coli* mid-log phase culture

Sample	Cell concentration (x 10^8 cfu/ml)		Fold increase
	Pre-incubation	Post-incubation	
Smaller cells	3.9	4.4	1.13
Mixed cells	2.6	4.4	1.69
Larger cells	1.5	4.3	2.87

results indicated that it is likely for larger *E. coli* cells to have a larger burst size.

DISCUSSION

The observation that the mixed sample cells were more likely to have greater burst size than the cells in the sample from the top of the band can be correlated to the cell sizes of those samples. This observation is supported by the results from Delbruck's burst size distribution experiment (2). As previously mentioned, we were successful in separating *E. coli* B23 cells based on size; therefore, the top sample should contain smaller cells while the mixed sample would contain a mixture of large, medium, and small cells. Larger cells have more protein synthesizing machinery (i.e. ribosomes) and therefore have a higher rate of T4 phage production (rise rate) (6). An increased rise rate results in larger burst sizes of T4 phage (6). In the top sample, smaller cells were present which would have less protein synthesizing machinery and result in a lower

TABLE 2. Summary of single burst sizes observed when *E. coli* samples recovered from different positions within the band after sucrose density gradient centrifugation were infected and incubated.

Sample	Number of plates with		Number of total plates
	0 plaque	> 0 plaque	
Smaller cells	32	8	40
Mixed cells	29	11	40
Larger cells	39	1[†]	40
Chloroform-treated mixed cells	39	1[†]	40

[†] plate contained a single plaque

Copyright © April 2010, M&I UBC

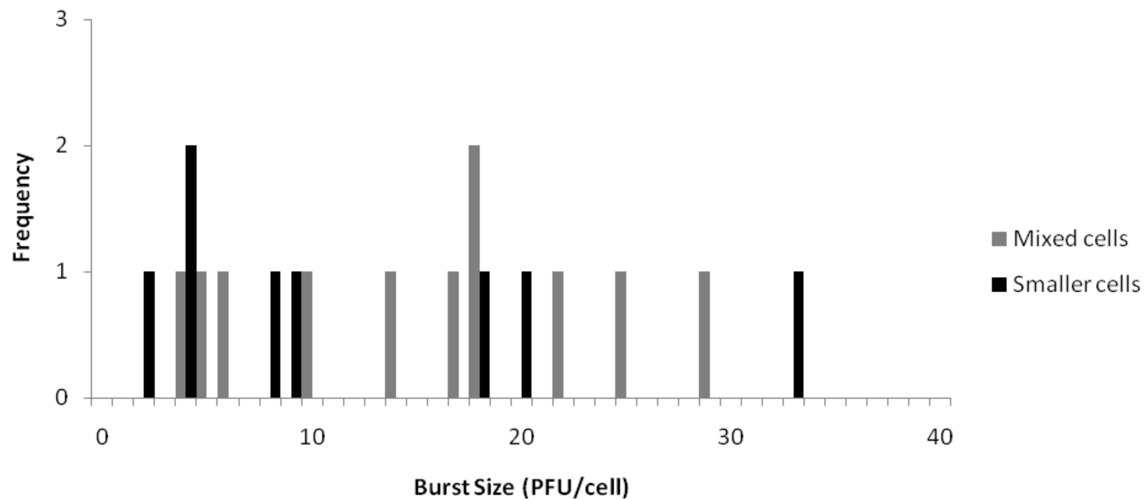

FIG. 3. The distribution of T4 burst sizes in infected *E. coli* cell samples enriched from smaller sizes and samples with normal distribution of mixed sizes. Burst size determined by assuming that each plaque-containing plate represented a single burst and that the number of plaques corresponds to the burst size.

rise rate and burst size. However, the average size of the cells in the mixed sample would be greater than the size of the cells in the top sample. Therefore, the cells in the mixed sample would have more protein synthesizing machinery, higher rise rates, and larger burst sizes as was observed in the single burst experiment.

None of the bottom cell sample plates had any results that showed a single burst of the virus. It is possible that none of the aliquots from the bottom sample showed a single T4 bacteriophage burst because phage were not released from the bottom sample cells before they were mixed with indicator cells and plated. You et al. found that in cells with larger volumes, there were higher numbers of *E. coli* RNA polymerases (EcRNAPs) and the EcRNAPs had an increased elongation rate (13). The increase in EcRNAP numbers and elongation rate resulted in a decreased rise rate of T7 phage infection of *E. coli* cells (13). The increase in EcRNAP numbers reduced procapsid synthesis of T7 phage which corresponded with a significant increase in number of ribosomes allocated to early T7 genes and a decrease in ribosomes allocated to late T7 genes (13). Early bacteriophage genes code for the scaffold protein, major capsid protein, and DNA maturation protein (6). Late bacteriophage genes code for late proteins which participate in phage particle formation and phage progeny release due to cell lysis (6, 13). It is possible that *E. coli* cells of the bottom sample had passed the critical size that contain large enough proportion of EcRNAPs to hinder the expression of late T4 genes. Therefore, in the bottom sample cells, the high amount of EcRNAP could have led to low levels of late T4 phage proteins which prevented or slowed down the rate of capsid formation and phage progeny release. This would potentially result in no phage progeny being released to infect the indicator cells and account for the lack of T4 phage burst from the cells of the bottom sample.

The low numbers of infected *E. coli* B23 cells may be due the presence of sucrose during the adsorption step. The experiment was done so that there would be 0.4 infected cells per 0.5ml aliquot of bacteria and phage mix. Forty plates were plated of each cell type and each would ideally have had 16 plates with infected cells. None of the groups had 16 infected cells. Sucrose contains a (1-3)-α-glucose-glucose linkage that may obstruct adsorption of phages to bacteria due to similarity of sucrose to the bacterial receptor portion for adsorption (5). T4 phage binds to a glucose residue at the distal end of the LPS in *E. coli* B strains (14). The possible inhibition of sucrose may be the reason why few bacterial cells were infected by the phage.

Normally the burst size of T4 phage of an individual burst is approximately 130 phages after 60 minutes of incubation at 37°C (1). Results seen in this experiment had burst sizes of 0-30 plaques per cell. After cells were separated by centrifugation, they were put on ice to prevent further growth and allow for time to determine

the cell concentration for each cell type. Once the cell concentration was determined, each type was mixed with T4 phage and allowed to adsorb. During the adsorption, the *E. coli* cells were previously on ice so the temperature was far below the optimal 37°C needed for optimal adsorption and phage development. *E. coli* cells have impaired growth at temperatures below 21°C and growth has stopped at 7.5^{o}C (10). During adsorption, the bacteria-phage mixture was warming up to room temperature for 10 minutes and then the mixture was placed in shaking water bath for 1 hour at 37°C. The temperature of the environment is known to play a factor in phage output. At 37°C the output of phage is approximately 130 phages per cell and at 42°C the output is 10 phages per cell after 60 minutes after infection (1). When the host is adjusting to the changes in temperature, it may not be in a physiological state that can support T4 phage production. Because *E. coli* had to adapt to the changing conditions of the environment, the bacteria may not have been optimal for phage production until the bacteria had adjusted to the 37°C environment. The delay in phage environment optimization may be a reason why phage counts were so low.

The single burst experiment results indicated that samples had mean burst sizes of 12 pfu and 15 pfu. However, these mean values are not statistically significant due to their large variances, which thwart powerful parametric tests such as the 2-sample T test. Therefore, the Mann-Whitney U test was used to relatively compare the distributions instead, which yields a weaker conclusion. On the other hand, Stent states that mathematical analysis of the model of intracellular phage growth shows that the high variance in number of phage progeny produced by individual cells is expected (9). Thus, the question becomes whether significance under the Mann-Whitney U test combined with Stent's observation is enough for supporting the obtained mean burst sizes. Furthermore, from Ellis and Delbruck's single burst experiment results, Stent observed that the average burst size can be calculated by dividing the total number of plaques by the total number of infected cells (9). Since the average burst size can be determined alternatively by the bacteriophage one-step growth curve, then it can justify the mean burst size values from the single burst experiment. Thus, the single burst experiment should be done simultaneously with the bacteriophage one-step growth curve for a more complete result and conclusion.

Based on our results we were successful in separating cells based on size and were able to draw the conclusion that size contributes to variations in the burst size. Furthermore, smaller cells have smaller burst sizes compared to the mixed population so larger cells likely have greater burst. However, it is probable that a critical cell size exists such that this correlation does not hold.

FUTURE DIRECTIONS

There were discrepancies in cell concentration determination where two methods were used to estimate concentration. Microscopic counts and plate enumeration of cells were done. For microscopic counts, cell dispersion is not as important and dead cells may be counted therefore there is a higher chance of overestimation of the cell concentration. For plate counts, it is important to disperse organisms so that one colony arises from one organism. If the bacteria are not dispersed efficiently, the cell concentration is underestimated. Also dead cells cannot make colonies, which contributes to the underestimation. Microscopic counts results for cell concentration were used for determining the concentration of phage for infection and dilutions needed for single cell bursts. Because the cell concentration obtained was an overestimation, the *E. coli* cells were diluted to a concentration that was lower than what was desired. So fewer cells were infected and fewer were plated than expected. In addition, cell enumeration after cell size separation was critical in terms of determining the correct dilutions schemes to perform the single burst experiment. Cell enumeration by direct microscopic count and the *B subtilis* conversion took a lengthy period of time. This delay may have affected cell sizes and concentration as the cells could have grown during enumeration which defeat the purpose of size separation as large cells could divide producing small cells. Several improvements in the current protocol could be implemented. The cell enumeration problem could be reduced by having three microscopes and chamber slides, so that each sample group (Mixed cell sample, Smaller cell fraction, Larger cell fraction) can be enumerated simultaneously which would minimize the delay. The effect of chilling should also be tested to determine whether these factors affect the burst and burst times. Another approach would be an attempt to elucidate the low burst size yield by comparing the average burst size obtained from the single burst experiment to the one step growth curve. Then the effect of sucrose on the bacteriophage infection can be investigated by comparing the burst size in the presence and absence of sucrose.

ACKNOWLEDGEMENTS

This study was funded by the Department of Microbiology and Immunology, University of British Columbia. The authors would like to thank Dr. William Ramey for his continual technical and theoretical support and for his dedication to exposing us to genuine scientific research. We would also like to thank our teaching assistant, Matt Mayer for his helpful suggestions and technical assistance. We would like to thank Jennifer Sibley for providing us with equipment and supplies. Finally, we would like to thank the media room staff for their assistance in preparing glassware and reagents.

RERFERENCES

1. **Camara, F. P. and D. F. de Almeida.** 1991. Studies on the growth of the phage T4 in cell division mutants DV2111 and AD651 of *Escherichia coli* at normal and high temperatures. Rev. Bras. Genet. **14**: 233-238.
2. **Delbrück, M.** 1945. The burst size distribution in the growth of bacterial viruses (bacteriophages). J Bacteriol. **50**:131–135..
3. **Ellis, E.L. and M. Delbrück.** 1939. The growth of bacteriophage. J. Gen. Physiol. **22**: 365-384.
4. **Giesa, F.R., I. Zajac, H.R. Bartus, and P. Actor.** 1982. Isopycnic separation of *Escherichia coli* cultures possessing colonization factor antigen I. J. Clin. Microbiol. **15**:1074-1076.
5. **Granboulan, P.** 1983. The tail fibre of bacteriophage T4 is sensitive to proteases at elevated temperatures. J. Gen. Microbiol. **129**: 2217-2228.
6. **Hadas, H., M. Einav, I. Fishov, and A. Zaritsky.** 1997. Bacteriophage T4 development depends on the physiology of its host *Escherichia coli*. Microbiology. **143**: 179-185.
7. **Karam, J. W., K. N. Kreuzer, G. Mosig, D. H. Hall, F. A. Eiserling, L. W. Black, E. K. Spicer, E. Kutter, K. Carlson, and E. S. Miller.** 1994. Molecular biology of bacteriophage T4. p. 429 - 431. American Society for Microbiology, Washington, D.C.
8. **Ramey, W. D.** 2009. Appendix D. *In* Microbiology 421 laboratory manual. University of British Columbia, Vancouver, BC.
9. **Stent, G. S**. 1963. Molecular biology of bacterial viruses. p. 74 – 77. W. H. Freeman and Company, San Francisco and London.
10. **Strocchi, M., M. Ferrer, K. N. Timmis, and P. N. Golyshin.** 2006. Low temperature-induced systems failure in *Escherichia coli*: Insights from rescue by cold-adapted chaperones. Proteomics. **6**: 193-206.
11. **Whitlock, M. and D. Schluter**. 2009. The analysis of biological data. p. 337 - 344. Roberts and Company Publishers, Colorado, US.
12. **Woldringh, C.L., J.S. Binnerts, and A. Mans.** 1981. Variation in *Escherichia coli* buoyant density measured in Percoll gradients. J. Bacteriol. **148**: 58-63.
13. **You, L., P.K. Suthers, and J. Yin.** 2002. Effects of *Escherichia coli* physiology on growth of phage T7 in vivo and in silico. J. Bacteriol. **184**: 1888-1894.
14. **Yu, F. and S. Mizushima.** 1982. Roles of lipopolysaccharide and outer membrance protein OmpC of *Escherichia coli* K-12 in the receptor function for bacteriophage T4. J. Bacteriol. **151**: 718-722